Lumi-Map, a real-time luciferase bioluminescence screen of mutants combined with MutMap, reveals Arabidopsis genes involved in PAMP-triggered immunity

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Abstract: Plants recognize pathogen-associated molecular patterns (PAMPs) to activate PAMP-triggered immunity (PTI). However, our knowledge of PTI signaling remains limited. In this report, we introduce Lumi-Map, a high-throughput platform for identifying causative single nucleotide polymorphisms (SNPs) to studying PTI signaling components. In Lumi-Map, a transgenic reporter plant line is produced that contains a firefly luciferase (LUC) gene driven by a defense gene promoter, which generates luminescence upon PAMP treatment. The line is mutagenized and the mutants with altered luminescence patterns are screened by a high-throughput real-time bioluminescence monitoring system. Selected mutants are subjected to MutMap analysis, a whole genome sequencing (WGS)-based method of rapid mutation identification, to identify the causative SNP responsible for the luminescence pattern change. We generated nine transgenic Arabidopsis reporter lines expressing LUC gene fused to multiple promoter sequences of defense-related genes. These lines generate luminescence upon activation of FLAGELLIN-SENSING 2 (FLS2) by flg22, a PAMP derived from bacterial flagellin. We selected the WRKY29-promoter reporter line to identify mutants in the signaling pathway downstream of FLS2. After screening 24,000 ethylmethanesulfonate (EMS)-induced mutants of the reporter line, we isolated 22 mutants with altered WRKY29 expression upon flg22 treatment (abbreviated as awf mutants). While five flg22-insensitive awf mutants harbored mutations in FLS2 itself, Lumi-Map revealed three genes not previously associated with PTI. Lumi-Map has the potential to identify novel PAMPs and their receptors as well as signaling components downstream of the receptors.

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